Isolation of Mouse Skin Cells with the Singulator™ Platform

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Introduction /

The skin is the outermost protective barrier organism and comprises of two main layers, the epidermis and the dermis. To understand skin homeostasis in healthy and diseased states, a thorough molecular characterization of every type of skin cell type is required. This in turn requires isolation of viable cells from skin for downstream genomics and cell biology analyses. However, traditional methods for isolating cells from skin tissue, are time-consuming, complex, and technically challenging. Here, we demonstrate the use of the Singulator™ 100 for isolation of viable mouse skin cells with high yield with S2 Genomics' Skin Cell Reagent.

Experimental Design /

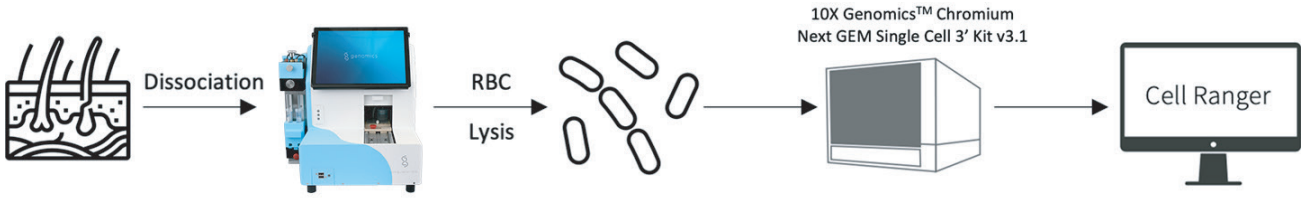

Figure 1. Experimental Design. Freshly harvested mouse skin tissue was dissociated into a single-cell suspension with a Singulator™ 100. The yield and viability of the cells were measured, a target number of cells were prepared with the 10X Genomics™ Next GEM Single Cell 3' kit v3.1. Sequencing data were analyzed with 10X Genomics' analysis pipeline and Seurat.

Methods /

Sample Processing

Whole skin tissue was excised from the dorsal side of a 6-week-old CD-1 mouse, trimmed of fur, rinsed, and finely minced into approximately 1-2 mm^2 pieces. Approximately 249 mg of fresh tissue was immediately processed in the Singulator 100 using the Mouse Skin Cell Isolation protocol, Mouse Skin Isolation Reagent (100-254-082), and Cell Isolation Cartridge (100-063-178). The resulting cell suspension was removed from the cartridge, centrifuged at 300 g for 5 minutes at 4°C, resuspended in 3 mL of DMEM, centrifuged at 300 g for 5 minutes at 4°C, and finally resuspended in 1 mL of DMEM. Cell counts and viability were then determined (AOPI staining, Nexcelom K2 (Revvity, Inc.)) to obtain the appropriate dilution for loading the sample onto the 10X Genomics Chromium Controller. The scRNA-Seq library was prepared using the 10X Genomics Next GEM™ Single Cell 3' kit v3.1 and sequenced.

Sequencing and Data Analytics

The skin tissue dissociation yielded approximately 5,200 cells per mg of tissue, with 90% viability. Images of the cells at 10x and 40x magnification are shown in Figure 2. The skin cell library was sequenced on an Illumina Novaseq™ 6000 by a third-party service provider. The data were analyzed with the 10X Genomics' Cell Ranger™ analysis pipeline and quality control filtering metrics were set to the manufacturer's default values. The data were independently clustered with Seurat v4.0 and the clusters were manually annotated using marker genes described in the literature[1,2].

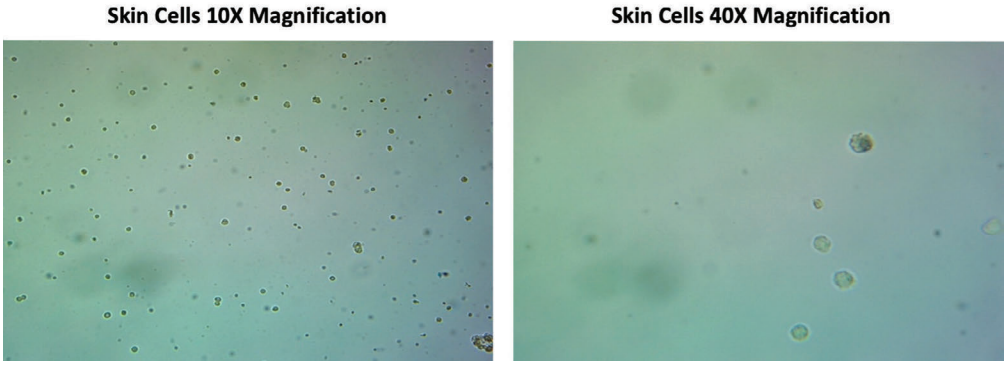

Figure 2. Skin Cells. Images of the cells at 10x and 40x magnification stained with Typan Blue for visual assessment of sample quality prior to sequencing library preparation.

Results /

Data for 6,851 mouse skin cells were analyzed and independently clustered with Seurat, annotated, and visualized as UMAPs. Approximately 2,302 genes per cell were detected with 20,092 mean reads per cell. The fraction reads in cells was 92.3%. High quality isolations were confirmed with 88% of the cells showing less than 10% mitochondrial contamination, as shown in Figure 3. Broad clustering shows all major cell types were isolated including Permanent Epidermal Keratinocytes, Fibroblasts, Anagen Hair Follicle Keratinocytes, T-cells, Immune Cells, Endothelial Cells, Schwann Cells and Melanocytes, and Skeletal Muscle Cells (Figure 4). Markers, such as Apoe, Gjb2, Krt15, and Fabp5 were used to identify the various keratinocytes. Dcn and Lum were used to identify fibroblasts, Sparc11 to identify muscle cells, and Mpz and Tyrp1 to identify melanocytes and Schwann cells. Tyrobp and Cd74 were used to identify immune cells, while Cd28 was specifically used to identify T-cells. Figure 5 shows the feature plots of these genes used for clustering of the cell types.

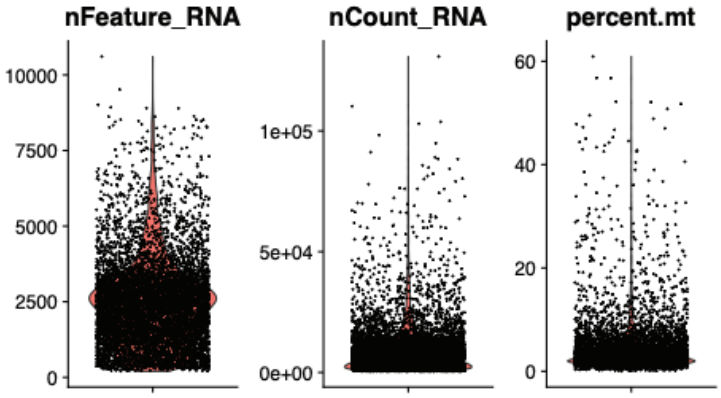

Figure 3. Violin Plots. Violin plots from scRNA-Seq data of skin cells from a 6-week-old CD-1 mouse isolated with the Singulator 100 showing nFeature RNA, nCount RNA, and percent mitochondrial contamination per cell.

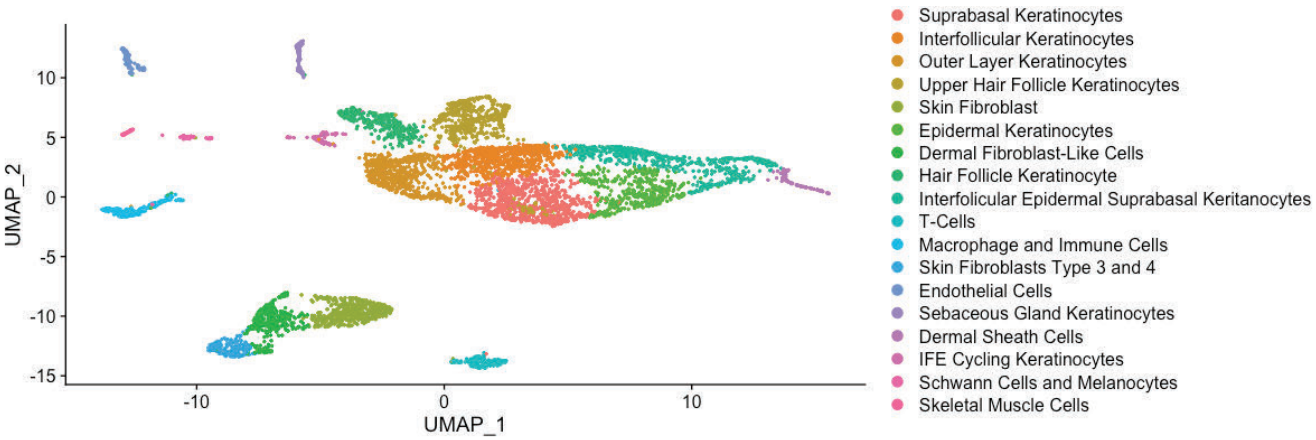

Figure 4. UMAP. UMAP from scRNA-Seq data of skin cells from 6-week-old CD-1 mouse isolated with the Singulator 100 showing broad clustering of cell types.

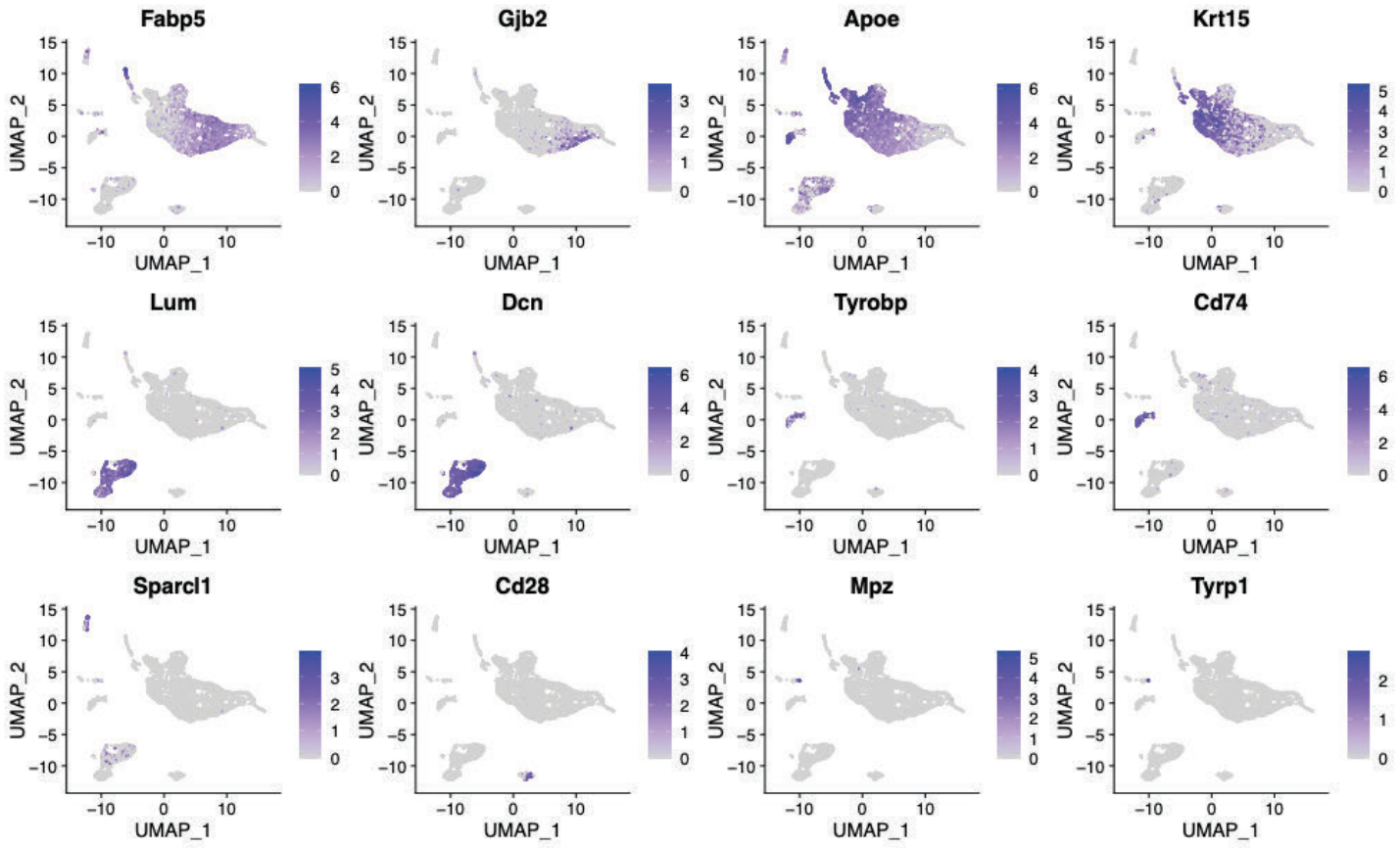

Figure 5. Gene Expression Feature Plot. UMAP with gene expression of various marker genes for the major cell types used to identify the various cell types in mouse skin tissue.

Conclusion /

Using the Singulator 100 system, we were able to successfully isolate viable skin cells and obtain single cell sequencing data with broad cell-type representation. The Singulator 100 eliminates the technical biases, time-consuming protocol development, and technical challenges associated with traditional cell isolation methods, allowing for improved cluster resolution and cell-type representation, including fragile cell types.

References:

1. Wang et al., Construction of a cross-species cell landscape at single-cell level. Nucleic Acids Research, 2022; gkac633, https://doi.org/10.1093/nar/gkac633.
2. Joost, Simon et al. The Molecular anatomy of Mouse Skin during Hair Growth and Rest. Cell Stem Cell, Volume 26, Issue 3, 441 - 457.e7 DOI: https://doi.org/10.1016/j.stem.2020.01.021